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STUDIES ON ENCEPHALITOZOON
III. RELATIONSHIP BETWEEN THE NATURAL INFECTION IN MICE AND THE ENVIRONMENTAL CONDITIONS

Keio Igaku (Journal of Keio Medical Society), Vol 37, 1960, pages 515-518.

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The author previously (1959) reported about the manifestation of Encephalitozoon in mice infected by the organism. The author made an investigation on the route of Encephalitozoon infection in laboratory mice, based on knowledge he had previously obtained and that of others. The route of Encephalitozoon infection has not been determined although many investigators reported that natural infection of the organism was widespread in many mammals and birds. Unlike Toxoplasma, which is an organism similar to Encephalitozoon, its virulence is very mild so that infected animals rarely die but maintain a state of chronic infection as previously reported. Therefore, an infected animal can be the source of infection for a long time. If the route of infection is clearly known, the infection may be prevented. Recently, Matsubayashi et al (1959) reported the presence of an infection in man, apparently caused by a similar organism. It is therefore very important to clarify the route of infection of this organism in order to contribute to further immunology and experimental zoology. It is for this reason that the author conducted the experiment of Encephalitozoon infection in laboratory mice under various environmental conditions.

Material and Method

The Encephalitozoon used in the experiment was isolated in 1956 from water rats and maintained intraperitoneally by passage through mice in author's laboratory. Mice as experimental animals were obtained from the market and they were apparently healthy and weighed about 15 grams each, indifferent as to sex. Six groups, each consisting of ten mice, were

separately kept under various environmental conditions and methods. Mice in group I were placed in the box which was used to keep mice infected by Encephalitozoon for a long time, and the box was placed in a sanitary separate room to prevent infection from other sources, since the box was obviously considered to be contaminated with excreta and food debris by Encephalitozoon infected mice. Group II was kept in a box which was sterilized by flame and the box was placed next to a box with mice infected by Encephalitozoon, in the animal laboratory. Group III was put in a sterilized box together with mice that were already infected by Encephalitozoon and placed in a separate sanitary room. Group IV was put into a sterilized box and each of them was injected with 0.5cc of urine from the Encephalitozoon infected mice and kept in a sanitary room. Group V, as in group IV, consisted of mice that were injected with 0.5cc of suspension of excreta of infected mice in 1:5 physiologic saline solution intraperitoneally. Group VI was kept in a sterile box and each of them was given 0.5cc of 10% emulsion of infected mouse liver and spleen into the stomach through a vinyl catheter twice daily.

Mice from each group were kept alive for three weeks and then killed for examination. The ascites was prepared for a hanging drop slide and examined under magnification of 1,000 times for the presence of organisms. The liver and spleen were prepared as 10% emulsion in a physiologic saline solution and it was injected intraperitoneally to healthy mice which were examined for the presence of organisms in their ascites after two and half weeks, in order to confirm the establishment of infection in the original mice. Mice that were infected by Encephalitozoon developed systemic parasitemia in two weeks or so after the infection and propagation of organisms is seen in ascites in three weeks or so, as previously reported. Identification of the organisms, therefore, is easily made by examination of ascites. Although there are no organisms found in ascites, there may be a period of systemic parasitemia and development of organ infection. It is for this reason that one prepares an emulsion of the liver and spleen of best affinity to the organism and it is passed into the peritoneal cavity of another mouse to identify the propagation of the organism.

It is most important in this experiment to know the existence of frequent natural infection of Encephalitozoon among mice, because the recognition of a newly established infection would be very hard in the presence of a pre-existing infection. This situation would be more difficult if the identification of the organism is made only after the passing of the liver-spleen emulsion of the original mouse to another mouse. Therefore, determination of infection in this experiment was made when the organisms were identified directly in experimental mice and also when many mice were positive in a particular group under various conditions.

Result

Mice in group I did not produce organisms in ascites after three weeks but intraperitoneal innoculation of the liver-spleen emulsion of

these mice to fresh mice produced the organism in one case. None was positive directly from ascites from group II, but one case was positive after the passage through fresh mice with emulsion intraperitoneally. Group III also showed no organisms in their ascites but two positive cases after passage with the emulsion. In group IV, six out of ten produced the organism in their ascites directly and in case of passage with their emulsions, the same six cases again showed positive and the other four remained negative. Group V did not produce organisms at all in either instance. Group VI also, as in group V, was totally negative.

Consideration

It is well known from various studies that Encephalitozoon is widespread among various animals. The author reported previously an observation on the course of this organism after entering the animal body. Possible sites of parasitic infection which may become the source of natural infection would be the organs with outward excretory passages such as blood, lymphatic fluid, oral cavity, salivary glands, intestine, kidney, and lung, etc., or some organs that could come in contact with various insect bites. Another possibility is the route through placental infection. As to blood, Kyo (1958), Iwami (1958), and the author reported that a fairly high concentration of the organism is present in two weeks after the infection up to six months. The organisms were present in the lung and kidney very early, as previously reported, especially in the kidney. From the standpoint of animals to be infected, the route of infection would be oral, percutaneous, mucosal -- such as respiratory organs, digestive organs, urinary organs, reporductive organs, and some of the sensory organs, and also direct injection by insect bites. In the author's experiments, the investigation was made to determine some of the possible routes of infection under possible environmental conditions. The experiment with group I was made under a contaminated environment only in the box. Although the box was contaminated with the feces and urine of the infected mice and also the contaminated food debris, the direct examination of ascites did not yield any organisms and only one positive case was seen in fresh mice that were injected with a liver-spleen emulsion made from the original mice. This fact indicates that the infection of Encephalitozoon is not easily established into the other mice even though they are kept in a heavily contaminated environment. It is likely therefore that the infection does not take place by simply having contact with contaminated materials on the skin or the ingestion of contaminated food, whereas the direct injection of infected urine intraperitoneally almost always, as in case of the experiment with group IV, resulted in establishment of infection. Natural infection then may be established over a long period of time when the affinity reaches an optimum between conditions of host and the organism. Group II yielded only one positive case after the passage of the emulsion to fresh mice. The establishment of infection in mice of group II that were kept in a disinfected box but placed side by side with another box in which infected mice were kept does not seem possible unless contamination by dust, transmission by blood sucking insects, of faulty feeding are responsible for it. In the case of group III, where the degree of contamination and contact with the organism is considered

to be very fresh and constant including the possible chances of biting each other, mating, and transmission by blood sucking insects, still none was positive in the examination of ascites and only two positive cases appeared after passage of the emulsion directly to fresh mice. This experiment was evaluated on the third week, therefore it is possible that the organism was not detected even in the presence of infection due to insufficient period of time, unless the infection took place at the beginning of the experiment. In group IV, the experiment was directed to determine the route of out-going organisms by injecting the urine of infected mice artificially into the peritoneal cavity, since the experiment with infected urine to establish the natural infection was covered in groups I and III. The result consisted of six positive cases out of ten mice. This fact indicates that the chance of infection from contaminated urine is highly possible. In the case of group V, the experiment was directed toward the establishment of infection with feces of infected mice which might contain the organism as in case of urine, but no evidence of excretion of organisms from the alimentary tract was noted. Presence of the organisms in the urine of infected mice is considered to be constant due to the fact that parasitemia and subsequent heavy kidney infections are usually present, but there seems to be no or only the rare presence of organisms in feces. The experiment with group VI was made to establish the infection based on the fact that the contaminated materials in the environment by excreta and food debris, etc., are obviously taken into body mainly by mouth. According to Iwami's report, the establishment of infection was seen in this fashion, although pH of gastric juice and the concentration of the organisms that were ingested with the material would effect greatly on the establishment of the infection, by instillation of contaminated materials into the stomach. No positive case was seen in this group of ten mice and it was thought that the establishment of infection in this fashion was not usually seen, even in an environment such as group I and group III, where a high degree of contamination from infected urine and food debris is obvious. An insignificant percentage of positive cases were seen in the organ passage into fresh mice, although the results might well be due to pre-existing natural infection either in the original mice or secondary inoculated mice, as reported by Koike et al in 1959. In this series of the author's experiments, it was confirmed that the organisms were excreted in urine and the establishment of infection, however, does not occur alone in the contaminated environment or from food contaminated by organism-infested urine, but there have to be various other factors also involved.

Conclusion

The experiments were made as to the establishment of natural infection of Encephalitozoon under various conditions.

2. No increase in infectivity was noted in this experiment under the contaminated conditions of the cage and its surrounding during the period of observation.

2. No increase in infectivity was noted in the experiment, in which known infected mice and healthy mice were kept together in the same cage during the period of observation.

3. Infective Encephalitozoon were excreted in the urine of infected mice.

4. Establishment of Encephalitozoon infection was not seem by innoculating the feces of infected mice into healthy mice.

5. Administration of the organisms by mouth did not establish infection.

In view of the above facts, the establishment of the natural infection is not seen under a condition contaminated by the excreted organisms alone, and there seemed to be certain additional contributing factors and chances that were necessary.

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Tables

Table 1

Group I (Contaminated Cage)

	1	2	3	4	5	6	7	8	9	10
Microscopic Examination of Ascites	-	-	-	-	-	-	-	-	-	-
Organ Emulsion Passage	-	-	+	-	-	-	-	-	-	-

Table 2

Group II (Sterilized Cage Placed Next to a Cage of Infected Mice)

	1	2	3	4	5	6	7	8	9	10
Microscopic Examination of Ascites	-	-	-	-	-	-	-	-	-	-
Organ Emulsion Passage	-	-	-	-	-	+	-	-	-	-

Table 3

Group III (Sterilized Cage with Coexistence of Healthy and Infected Mice)

	1	2	3	4	5	6	7	8	9	10
Microscopic Examination of Ascites	-	-	-	-	-	-	-	-	-	-
Organ Emulsion Passage	-	-	-	-	-	-	+	-	+	-

Table 4

Group IV (Sterilized Cage and Innoculation of Infected Mouse Urine)

	1	2	3	4	5	6	7	8	9	10
Microscopic Examination of Ascites	+	+	-	+	-	+	+	+	-	-
Organ Emulsion Passage	+	+	-	+	-	+	+	+	-	-

Table 5

Group V (Sterilized Cage and Innoculation of Infected Mouse Feces)

	1	2	3	4	5	6	7	8	9	10
Microscopic Examination of Ascites	-	-	-	-	-	-	-	-	-	-
Organ Emulsion Passage	-	-	-	-	-	-	-	-	-	-

Table 6

Group VI (Sterilized Cage and Intra-gastric Instillation of Infected Organ Emulsion)

	1	2	3	4	5	6	7	8	9	10
Microscopic Examination of Ascites	-	-	-	-	-	-	-	-	-	-
Organ Emulsion Passage	-	-	-	-	-	-	-	-	-	-